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Cytogenetic Analysis of 750 Spontaneous Abortions with the Direct-Preparation Method of Chorionic Villi and Its Implications for Studying Genetic Causes of Pregnancy Wastage

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Summary

Altogether, 750 cases of spontaneous abortion between the fifth and 25th week of gestation were analyzed cytogenetically by the direct-preparation method using chorionic villi. The majority of cases (68%) were derived from early abortions before the 12th week of gestation. The frequency of abnormal karyotypes was 50.1%; trisomy was predominant (62.1%), followed by triploidy (12.4%), monosomy X (10.5%), tetraploidy (9.2%), and structural chromosome anomalies (4.7%). Among trisomies, chromosomes 16 (21.8%), 22 (17.9%), and 21 (10.0%) were prevalent. The frequency of chromosomally abnormal abortions increased with maternal age but only because of an increase of trisomy. Polyploidy and monosomy X, however, decreased. Mean maternal age was significantly increased for trisomies 16, 21, and 22 and was highest for trisomies 18 and 20. The results obtained are within the range of variability reported earlier from tissue culture–type studies. A consistent feature during our study is the excess of females in chromosomally normal abortions (male:female sex ratio 0.71). According to the methodology applied, maternal cell contamination and undetected 46,XX molar samples cannot have influenced the sex ratio. However, a bias introduced by social status or maternal age cannot be excluded. With the more rapid and convenient direct preparation of chorionic villi, reliable cytogenetic data on causes of spontaneous abortions can be obtained.

Introduction

Fecundability in man, in comparison with other mammals, is rather low and is estimated to be 21%–28% at an age of 20–30 years (Short 1979). The diminished rate is explained by an enormous frequency of pregnancy wastage, either before or during the implantation phase. Later on, in clinically recognized pregnancies, a further 15% of conceptuses are lost as spontaneous abortions between the sixth and 28th weeks of gestation (Kline and Stein 1985). The majority of early pregnancy wastage is caused by karyotypic abnormalities such as polyploidy, trisomy, and monosomy X (Boué et al. 1985; Jacobs and Hassold 1987). This inference can be made from several cytogenetic studies performed after the initial reports on the frequent occurrence of chromosome anomalies in early abortions (Carr 1963, 1965). Other genetic causes are the suggested influence of parental HLA sharing (Thomas et al. 1985), mutated early developmental genes (Rossant and Joyner 1989), and situations of noncomplementation by uniparental disomy (Searle and Beechey 1985). The cytogenetic classification of spontaneous abortions is a prerequisite for investigation of such nonkaryotypic but genetic causes of pregnancy wastage.

Furthermore, karyotyping abortuses is important clinically and useful for therapeutic (Adinolfi 1986; Mowbray et al. 1987) as well as for prognostic reasons

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(Warburton 1985; Morton et al. 1987; Warburton et al. 1987). Such an analysis also provides information on the frequency and type of chromosome anomalies in different populations and on their etiology and recurrence risks (Hassold et al. 1980; Kajii et al. 1980; Warburton et al. 1980; Yamamoto et al. 1982; Hassold and Jacobs 1984; Warburton 1985; Hassold 1986; for review, see Hansmann et al. 1989).

A routine cytogenetic analysis of abortions has been hindered so far by the necessity for conventional tissue culture. This technique is laborious and afflicted with the risk of contamination, culture failure, and selective growth of maternal cells. Karyotyping is also not replaceable by the histological examination of the placenta, because of the low predictive value of chorionic villi histology (Minguillon et al. 1989; Rehder et al. 1989).

By means of a modified technique of analyzing chromosomes directly from chorionic villi (Simoni et al. 1983), it was demonstrated recently that early abortions can be karyotyped without resorting to tissue culture (Eiben et al. 1986; Hansmann et al. 1986). Before this simpler method could be used as a routine procedure, it was necessary to evaluate the significance of its experimental data in comparison with those obtained by tissue culture–type studies.

Material and Methods

Cytogenetic analysis of 750 of 983 consecutive spontaneous abortions was successfully performed by the direct-preparation method using chorionic villi (76.3%). With respect to maternal and gestational age, the 233 (23.7%) samples in which no information on the karyotype was obtained did not differ significantly from the 750 karyotyped abortions. In such cases, autolysis of villi, an insufficient amount (i.e., less than 5 mg) of chorionic villi, or errors during transportation were considered to be responsible for the failure of the cytological preparation. Samples were obtained from the University Hospital, Göttingen (Professor W. Kuhn); the Albert-Schweizer-Krankenhaus, Northeim (Professor R. Rauskolb); and the Evangelische Krankenhaus, Oberhausen (Professor R. Goebel). Gestational age was established by ultrasound.

Within 1 d the samples were sent in culture medium to the Institute of Human Genetics, Göttingen, (417 cases) and to the Institute of Clinical Genetics and Cytology, Oberhausen (566 cases). In both laboratories the material was handled according to a standardized procedure described elsewhere (Eiben et al. 1986). Shortly thereafter, the samples were examined microscopically at 8–10 × magnification, maternal tissue was removed, and 5–50 mg chorionic villi were selected for incubation. Incubation was performed in Petri dishes containing 5 ml prewarmed α-MEM supplemented with 10% basal medium supplement (Seromed; Berlin), 10% Condimed (Böhringer, Mannheim), 1% streptomycin/penicillin, and 1% glutamine for approximately 24 h at 37°C and 5% CO_2. Colchicine (0.04 ng/ml) was added 2 h before cytological preparation. Metaphases were obtained by following a modified method of Simoni et al. (1983). At least five metaphases per case were analyzed after solid Giemsa staining and Q- or trypsin-Leishman banding. When required, C-banding or silver staining was also performed. All the data obtained were subjected to Student's t-test and to the χ^2 test. A probability level of .05 was accepted as the criterion for a statistically significant difference.

Results

Frequency and Type of Chromosome Anomalies

The results from both laboratories did not differ significantly from each other, and the data are summarized in the following. The overall frequency of chromosome anomalies among the 750 karyotyped abortions was found to be 50.7%. When abortions with hydatidiform moles (n = 4) with a 46,XX karyotype were excluded, the frequency was 50.1% (table 1). The majority (68%) of cases were derived from abortions occurring before the 12th week of gestation and showed the highest frequency (54.1%) of abnormal karyotypes. In the smaller group of abortions, occurring after the

Table 1

Gestational Age, Number of Abortions, and Frequency of Pathological Karyotypes

Week of Gestation	No. of Abortions Karyotyped	% of Chromosomally Abnormal Abortions
5–7	50	50.0
8–9	142	51.4
10–11	300	56.0
12–13	142	50.0
14–15	55	38.2
≥16	35	22.9
Unknown	26	38.5
Overall	750	50.1[a]

[a] Hydatidiform moles excluded.

Table 2

Karyotypes of 750 Spontaneous Abortions, by Gestational and Maternal Age

Karyotype	No. of Abortions	Mean ± SEM Gestational Age (wk)	Mean ± SEM Maternal Age (years)
Normal	370	11.3 ± 2.7	28.8 ± 6.1
Abnormal:	380	10.7 ± 2.3	31.6 ± 5.9
Polyploidy:			
3n	46	11.5 ± 2.3	28.4 ± 5.0
3n + 1	1		
4n	16	11.3 ± 1.4	29.8 ± 5.3
4n + 1	1		
4n/2n	16		
4n/2n + 1	2		
Trisomy:			
All	236	10.6 ± 2.6	33.5 ± 6.3
Single	229	10.6 ± 2.6	33.5 ± 6.2
Double/triple	7	9.6 ± 1.1	35.1 ± 9.6
Monosomy X	40	10.9 ± 2.0	28.0 ± 6.0
Structural anomaly	18	8.9 ± 1.5	27.8 ± 5.4
Hydatidiform mole	4		

11th week of gestation (32%), chromosome errors were detected at a lower frequency (44.1%). Trisomy was the predominant chromosome anomaly and accounted for 62.1% of all abnormal abortions, followed by polyploidy (21.6%, abortions with 2n/4n mosaicism included), monosomy X (10.5%), and structural abnormalities (4.7%) (table 2).

Among the triploid abortions the gonosomal constitution of XXY prevailed (n = 28), followed by XXX (n = 15) and XYY (n = 3). One case of these triploid abortions showed mosaicism with an extra chromosome 6 (70,XXX,+6) and with an additional second cell line with 70,XXX,+6p$^-$.

Tetraploidy amounted to 9.2% among chromosomally abnormal abortions, including 18 cases with 2n/4n or 2n+1/4n mosaicism (table 2). The frequency of tetraploid metaphases in these cases was 20%–80%. The two abortions with the exceptional 2n+1/4n mosaicism were trisomic, in the diploid cell line, for chromosomes 13 (47,XX,+13/92,XXXX) and 16 (47, XY,+16/92,XXYY), respectively.

Trisomies for all chromosomes, with the exception of chromosomes 1, 5, 17 and 19, were observed. Chromosomes 16 (21.8%, without double and triple trisomies), 22 (17.9%), 21 (10.0%), and 13 (8.7%) were most frequently involved in trisomy (table 3). Altogether, six cases with trisomy for two chromosomes (+4+14; +11+21;+13+16;+13+22;+13+D; and +21+22) and one case with trisomy for three chromosomes (+D+E+G) (3.0% of all trisomic abortions) were found.

All 18 abortions with a structural chromosome abnormality had an unbalanced karyotype. These unbalanced karyotypes were due to translocation (n = 15) or deletion (n = 3).

Maternal Age

Mean (± standard error of the mean [SEM]) maternal age was lowest for abortions with structural anomalies (27.8 ± 5.4), monosomy X (28.0 ± 6.0), and polyploidy (28.4 ± 5.0 for triploidy and 29.8 ± 5.3 for tetraploidy) when compared with other chromosomal aberrations. These mean maternal ages are, however, not significantly different from that for chromosomally normal abortions (28.8 ± 6.1; table 2). Only for trisomic abortions was maternal age significantly increased (33.5 ± 6.3). Significant differences were observed when trisomies of individual chromosomes were compared with regard to mean maternal age. Mean maternal age was lowest for trisomies of group A-B chromosomes (28.8 ± 4.6), was significantly higher for trisomies of chromosomes 16 (33.1 ± 4.6) and 21 and 22 (35.1 ± 4.9), and was highest for trisomy of chromosome 18 and 20 (38.8 ± 6.1) (table 3).

The frequency of abnormal karyotypes differed among the samples allocated to the five different maternal age groups, as shown in table 4. The frequency increased significantly, from 40.0% in the ≤24 years age

Table 3

Type of Trisomy, by Gestational and Maternal Age

Trisomy for Chromosome	No. of Abortions	Mean ± SEM Gestational Age (wk)	Mean ± SEM Maternal Age (years)
2	3	12.8 ± 4.7	28.8 ± 4.6
3	7		
4	7		
6	6	9.3 ± 1.9	31.8 ± 1.8
7	8		
8	4		
9	6		
10	4		
11	4		
12	6		
C	1		
13	20	10.9 ± 2.0	32.2 ± 6.4
14	5		
15	7		
D	1		
16	50	9.7 ± 2.0	33.1 ± 4.6
18	15	11.5 ± 2.5	38.8 ± 6.1
20	11		
21	23	11.4 ± 2.0	35.1 ± 4.9
22	41		
Double	6	9.6 ± 1.1	35.1 ± 9.6
Triple	1		
Overall	236	10.6 ± 2.6	33.5 ± 6.3

group to 82.2% in the ≥40 years age group. This is exclusively due to an increase of trisomic abortions. While trisomic abortions increased nearly sixfold with age, monosomy X and polyploidy decreased significantly. The frequency of structural anomalies did not change with maternal age.

Sex Ratio

The overall sex male:female ratio among the 370 spontaneous abortions with a normal 46,XY and 46,XX karyotype was 0.71. This altered sex ratio in favor of chromosomally normal female abortions is significantly different from the ratio of 1.06–1.07 ($P < .01$) in the newborn population (Visaria 1967), from the ratio of 1.17 ($P < .0027$) in normal first-trimester pregnancies monitored by chorionic villus sampling (Bartels et al. 1990), and from the ratio of 1.16 ($P < .001$) in induced abortions analyzed similarly by the direct-preparation method using chorionic villi (Zhou et al. 1989).

Table 4

Frequency and Type of Chromosome Anomalies in Spontaneous Abortions, by Maternal Age

Maternal Age (years)	Mean ± SEM Gestational Age (wk)	No. of Abortions	% of Abnormal Abortions	% of Abnormal Abortions with			
				Polyploidy	45,X0	Trisomy	Other
≤24	11.1 ± 2.2	140	40.0	12.9	10.0	14.3	2.9
25–29	11.4 ± 3.2	242	40.5	12.8	4.6	21.5	1.7
30–34	10.8 ± 2.5	184	55.4	11.4	3.8	37.5	2.7
35–39	10.9 ± 2.4	118	67.8	10.2	5.9	48.3	3.4
≥40	10.5 ± 2.1	45	82.2	.0	.0	80.0	2.2
Unknown		21	33.3	.0	4.8	9.5	19.1
Overall	11.0 ± 2.5	750	50.7	10.9	5.3	31.5	2.9

Table 5

Sex Ratio in Chromosomally Normal Spontaneous Abortions, by Gestational Age

Gestational Age (wk)	Male	Female	Ratio
5–9	35	58	.60
10–13	94	109	.86
≥13	20	39	.51
Overall	149	206	.72

Table 6

Sex Ratio in Chromosomally Normal Spontaneous Abortions, by Maternal Age

Maternal Age (years)	Male	Female	Ratio
≤25	40	53	.76
26–35	86	120	.72
≥36	20	25	.80
Overall	146	198	.74

Mean maternal age was 28.3 ± 5.3 years for male abortions and 29.0 ± 6.2 years for female abortions. On average, male abortions occurred at week 11.1 ± 2.8, and female abortions occurred at week 11.4 ± 3.0. The four hydatidiform moles with a 46,XX chromosome constitution are not included in these data. Samples from all abortions were analyzed simultaneously by two pathologists (Minguillon et al. 1989; M. Vogel, personal communication), and no additional case of a mole with 46,XX was recorded. It is unlikely, therefore, that unrecognized 46,XX molar samples could have contributed to the excess of chromosomally normal female abortions.

The relationship between gestational age and sex ratio is shown in table 5. The ratio was lower in early and late abortions than in abortions between 10 and 13 wk. However, these differences are statistically not significant. The relationship between maternal age and sex ratio is given in table 6. No significant difference was observed.

The overall male:female sex ratio of 236 spontaneous abortions with autosomal trisomy is 0.95. The mean gestational ages are 10.2 ± 2.1 years for males and 11.0 ± 2.8 years for females. Mean maternal age was calculated to be 33.3 ± 5.8 and 33.8 ± 5.7 years for males and females, respectively, and is significantly ($P < .05$) higher than that of chromosomally normal male and female abortions.

Discussion

Influence of Methodology on Cytogenetic Results

The results of the present study provide strong evidence for a general applicability of the chorionic villi method for karyotyping spontaneous abortuses directly from the placenta. The frequency and type of chromosome anomalies detected in our study is very similar to data of previous investigations using conventional tissue culture (table 7). The apparent similarity between our observations and those from five comprehensive studies using tissue culture of heterogeneous tissues, such as tissue originating from a fetus, umbilical cord,

Table 7

Frequency and Type of Chromosome Anomalies in Spontaneous Abortions Karyotyped, after Conventional Tissue Culture and After Direct-Preparation Method Using Chorionic Villi

Method and Study	No. of Abortions Karyotyped	% of Abortions						
		Abnormal	Triploid	Tetraploid	Trisomic	45,X0	Structural Anomalies	Other
Tissue culture:								
Boué et al. 1985	1,498	61.5	12.2	3.8	33.0	9.4	2.3	.7
Creasy et al. 1976	941	30.5	4.0	1.3	16.3	7.2	1.2	.5
Kajii et al. 1980	402	53.5	5.2	1.7	32.6	10.5	2.7	.8
Warburton et al. 1980	967	32.3	5.4	2.0	18.0	5.0	.9	1.0
Hassold 1986	2,919	50.5	6.9	2.5	29.1	9.1	2.4	.7
Chorionic villi:								
Present study	750	50.7	6.3	4.7	31.5	5.3	2.4	.5

amnion, or chorion (Creasy et al. 1976; Hassold et al. 1980; Kajii et al. 1980), is highly significant. The results obtained with the direct-preparation method are within the range of variability reported for the tissue culture–type studies and resemble those from Hassold et al. (1980) and Kajii et al. (1980) (table 7). However, three major differences between the results from the two methods are apparent—namely, the relation between trisomy and monosomy X, the kind of trisomy, and the frequency of tetraploidy. The first two differences can be explained by a selection bias, by temporal changes in rates of chromosome anomalies (Hassold and McLean 1984), or by differences in mean maternal age (Hassold and Chiu 1985), which in our study is 2–3 years older.

Methodological reasons, however, seem to be responsible for differences in the frequency of tetraploidy (table 7). It is remarkable that approximately half of our tetraploid abortuses are 2n/4n mosaics with tetraploidy present in more than 20% of the cells analyzed. In tissue culture–type studies the frequency of such abortions having 2n/4n mosaicism is much lower, e.g., 1.1% in the study by Hassold et al. (1980). If only the data on abortuses with complete tetraploidy were calculated, the frequency in our study would be 2.3%, which is in the range of tetraploid abortions in the earlier studies (table 7).

Polyploid metaphases are an inherent feature of cell culture, and they arise as pseudomosaicism in cells from nearly every tissue, including that of fetal and placental origin with a proven constitutive diploid karyotype (Hunt and Jacobs 1985*a*). Placental cultures seem to be particularly prone to clonal growth of chromosomally abnormal cells, and tetraploidy developed preferentially in placental cultures from spontaneous abortions (Hunt and Jacobs 1985*a*, 1985*b*). It is obvious, therefore, why such a diploid/tetraploid mosaicism has heretofore been classified as an artifact arising during cell culture of spontaneous abortions. Cultural artifact, however, is an unlikely explanation for 2n/4n mosaicism in the 18 abortions of the present study. In all cases metaphases were prepared immediately after a short incubation period of approximately 24 h. Moreover, when the same procedure was used, tetraploid metaphases were detected only occasionally in a few ongoing pregnancies monitored by chorionic villus sampling during the first trimester (authors' unpublished results). Furthermore, all 2n/4n abortions were classified independently by chorionic villus histology as being chromosomally abnormal, and their recorded histological phenotypes did not differ from those of abortions having only tetraploid metaphases (Minguillon et al. 1989; M. Vogel, personal communication). The histological phenotypes included irregular width with smooth villous surface and some intrastromal cytotrophoblast cells, focal appearance of large hydropic villi together with an irregular branching pattern, and an absence of stem villi differentiation (Minguillon et al. 1989).

It is reasonable to assume that a significant number of early spontaneous abortions are characterized by placental mosaicism with diploid and tetraploid cells detectable only by the direct-preparation technique. Such a mosaicism might result from a disordered placental development ending in an abortive pregnancy or might even cause the abortive event. Kalousek and Dill (1983) described confined chorionic mosaicism for trisomies in chromosomally normal conceptuses having intrauterine growth retardation (IUGR). They suggested that such pregnancies may develop up to term—with, e.g., IUGR as a manifestation of placenta malfunction. Another form of confined placental mosaicism having only normal metaphases in cytotrophoblasts also has been described, and this type seems to facilitate intrauterine survival of trisomic conceptuses (Kalousek et al. 1989). When the present method of analysis was used, no evidence for other placental mosaicism was found in our samples of spontaneous abortions.

Sex Ratio in Chromosomally Normal Abortions

The altered male:female sex ratio (0.71), with an excess of females in chromosomally normal abortions, in remarkable. The finding is in contrast to the estimated male:female sex ratio of 1.32 calculated from tissue culture–type studies (Hassold et al. 1983). As per the direct-preparation method and histological examinations, however, our ratio can be explained neither by maternal cell contamination nor by an undetected contribution of 46,XX abortions from molar samples. Moreover, the ratio did not fluctuate considerably during the course of our study and was calculated to be 0.76 for the first 140 abortions (Eiben et al. 1987) and 0.77 for 500 abortions (Bartels et al. 1990). The excess of females is also not restricted to abortions from a single geographical area. The ratio is 0.72 in the sample of 270 abortions studied in the more rural area of Göttingen. Among the 480 abortions from a more urban area around Oberhausen the sex ratio is calculated to be 0.71. Since no information on the social background is available, the possibility of these factors biasing our sample cannot be ruled out. Sex ratio might have been influenced also by the higher mean maternal age in our study: the mean maternal age in our study

is 2–3 years older than that in the earlier studies, which used tissue culture. However, our data show no significant influence of maternal age on the sex ratio (table 6). The altered sex ratio could also have arisen from a biased and sex chromosome–specific failure of chromosome preparation—approximately 70%–90% of all samples were karyotyped successfully—particularly with abortions having a 46,XY karyotype.

Biological causes for an altered sex ratio, in favor of females, in chromosomally normal abortions are also likely and have been discussed recently (Bartels et al. 1990). It was hypothesized that failures during in- or reactivation of the X chromosomes could generate an abnormal function of X chromosomal and/or autosomal genes at very early stages of embryonic and placental development which later on may result in an abortive pregnancy. If corroborated, our data would provide the first evidence for a female-specific developmental disadvantage at early stages of gestation, a developmental disadvantage which would be in opposition to the later, male-specific peri- and neonatal mortality (Scheinfeld 1958; Machin and Crolla 1974; Sutherland et al. 1978).

In conclusion, the cytogenetic analysis of 750 spontaneous abortions by the direct-preparation method using chorionic villi furnished results which are similar to those obtained previously with tissue culture–type studies. The frequency and type of chromosome anomalies are directly comparable to those of other reports. Minor differences recorded are explainable by differences between mean maternal and gestational ages. The only discrepant results which might be ascribed to the method of direct preparation concern (*a*) the detection of significant numbers of abortions with 2n/4n placental mosaicism and (*b*) the altered sex ratio with an excess of females. Altogether, this method is advantageous in several respects, while it is less laborious and provides a rapid cytogenetic diagnosis. Among other advantages, its routine application would contribute to the identification of mutated early developmental genes (Rossant and Joyner 1989) causing pregnancy wastage of chromosomally normal conceptuses.

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